ON THE TREATMENT OF TRYPANOSOMIASIS BY ATOXYL (AN ORGANIC ARSENICAL COMPOUND), FOLLOWED BY A MERCURIC SALT (MERCURIC CHLORIDE) BEING A BIO-CHEMICAL STUDY OF THE REACTION OF A PARASITIC PROTOZOON TO DIFFERENT CHEMICAL REAGENTS AT DIFFERENT STAGES OF ITS LIFE-HISTORY

By BENJAMIN MOORE, M.A., D.Sc. (R.U.I.), *Johnston Professor of Bio-Chemistry;* MAXIMILIAN NIERENSTEIN, PH.D. (BERNE), *International Research Fellow;* AND JOHN L. TODD, B.A., M.D., C.M. (McGILL), *Director of the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine.*

From the Johnston Laboratories of Bio-Chemistry and of Tropical Medicine, University of Liverpool

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The experiments recorded below are of interest, first, from their special application to the prevention of the recurrence of trypanosomiasis after the parasites have been attacked and driven out of the blood by a remedial agent; and, secondly, from the more general point of view as an illustration of the important fact that when dealing with a parasitic protozoon which is attacked by a chemical substance or drug at one definite period only of its existence, it may happen that the drug may cause the parasite to pass into an encysted or a passive form which is no longer attacked by the drug and which, when conditions in the host have changed, may give rise to a fresh development of the parasite in its active form and a consequent recurrence of the disease. Further, the experiments show that a second substance used subsequently as a therapeutic agent may attack the passive form of the parasite and destroy it although this second agent may have no effect whatever upon the first or active form of the parasite and used alone as a curative agent would be entirely without effect.

1. A preliminary notice on this subject by us appeared in the *Annals of Tropical Medicine and Parasitology*, Vol. I, No. 1, p. 161, March, 1907.

It is already well known that in certain diseases due to protozoa a drug attacks the parasite at one period of its life cycle and is powerless at other times, as, for example, in the action of quinine upon the parasite of malaria.

Empirically discovered instances are also known where one drug is efficient at an early stage of a disease, and another drug is specific at a later stage, although our knowledge is not yet sufficiently advanced to enable us to state whether or not this reaction of the disease-producing parasite to different drugs at different stages corresponds definitely to different stages of development of the parasite at these respective periods.

Thus in the case of syphilis, which has recently been shown to be caused by a protozoon, mercury is specific in its action in the earlier stages while iodides are without effect; at a later stage, while mercury is still indicated, used alone it is insufficient, and the iodides show a well marked specific action.

It was reasoning on such lines which induced us to attempt by empirical experimentation to discover some drug which should prevent the recurrence of trypanosomiasis after the trypanosomes had been driven out of the blood by some drug, such as atoxyl, which possesses a specific action upon the usual active form in which the trypanosome is found in the blood and tissues.

The results hitherto obtained by experimenting with mercuric chloride in rats experimentally infected with nagana (*T. brucei*) from which the trypanosomes had been driven out of the blood by treatment with atoxyl, have been sufficiently encouraging to induce us to publish them, as an example of this principle of experimenting with a view to finding reagents capable of attacking protozoa at different stages. Our findings may perhaps encourage experimentation along similar lines in the case of other protozoa.

Various substances have been described by different investigators which possess the power in varing degree of driving trypanosomes out of the blood of animals infected with different forms of trypanosomiasis.

These substances when they have been demonstrated to have an

action upon one species of trypanosome have usually been shown later to affect the other well-known species of trypanosome also, although quantitatively the effect varies somewhat from species to species. Accordingly, with certain quantitative restrictions, trypanosomiasis may be regarded as a whole, and from the therapeutic point of view, one species may be taken as a sample in the first instance, although afterwards naturally the results so obtained must be extended by experimentation to the other important species.

As a material for experimentation, the *Trypanosoma brucei* presents many advantages, in the ease with which infection can be passed from animal to animal; the rapidity with which development occurs in the usual laboratory animals, and the comparatively short interval in which in the vast majority of cases the parasites recur again after treatment with the initial drug has been given and interrupted. On the other hand, the long latency of infection and development, and also after initial treatment the slow and irregular recurrence of the parasite, which may not take place for months or years, render the trypanosome of sleeping sickness (*T. gambiense*) tedious and uncertain for such work of testing the effect of a second drug.

Accordingly, we have used for our experiments chiefly a strain of *T. brucei*, but are now engaged in extending our observations to the trypanosome of sleeping sickness. Also in order to be able to carry out a large number of experiments we have used small animals (rats) but are now proceeding to experiment with guinea pigs, rabbits, dogs, and asses.

The remedies which have up to the present been shown to possess the effect of, at least initially or temporarily, driving trypanosomes out of the blood, may be divided into two classes, viz., (*a*) certain aniline colouring matters which possess a similarity in molecular constitution—of these the most effectual are trypanroth (Ehrlich and Shiga) (1) and p. di-amido-di-phenyl-urea + Ac.H [Ph.], p. di-chloro-benzidine + Ac.H. [Cl] (Mesnil, Nicolle, and Aubert) (2),[1]

1. These two colouring materials are supplied by the firm, Farbenfabriken vorm. Bayer & Co., Elberfeld, under the names, Afridol violet, and Afridol blue. It is a pleasure to express our indebtedness to Messrs. Bayer & Co. for their kindness in presenting us with quantities of these and other colouring matters for use in our researches.

and malachite green (Wendelstadt) (3); and (*b*) compounds of arsenic of which the most effectual by far is the organic aniline derivative known as atoxyl.[1]

We have, up to the present, used only atoxyl as the initial drug for driving out the parasite from the blood, but are at present investigating the effect of using the aniline colour preparations mentioned above for this purpose.

The beneficial action of arsenical compounds has been known for many years, having been first pointed out by the explorer Livingstone in regard to nagana. Since then many observers (Bruce, Lingard, Laveran and Mesnil, Thomas and Breinl, and others) have recorded the marked action of various forms of arsenical compounds administered in different ways, on the parasites in the blood stream of animals infected with trypanosomes of several different species.

It has been universally recognised, however, that in the vast majority of cases the protective effect of the usual forms of arsenic is only temporary and that, in the end, the parasites reappear in spite of steadily increased doses of arsenic until a point is reached at which the animal dies either of the disease or through poisoning by the arsenic.

A great advance was made on the treatment by ordinary forms of arsenic by Thomas alone, and in collaboration with Breinl (4). These authors introduced treatment by atoxyl, an organic compound of aniline and arsenic. This discovery was due to a search amongst the organic compounds of arsenic for one which would still retain its deleterious effect on trypanosomes but would be less toxic to the animal treated. A number of preparations were tried without result until atoxyl was found to possess both of the desired qualities to a marked degree. A large series of experiments on animals infected by various species of trypanosomes was carried out at the Runcorn Laboratory. It was found that the trypanosomes could be rapidly driven out of the blood by atoxyl, and that this substance was much more potent as a remedy than any other therapeutic agent hitherto

1. Sold by Lanolinfabrik Martinikenfelde, Berlin.

described, and it may here be added than any since described. As a result of their experiments, Thomas and Breinl recommended strongly the systematic treatment of sleeping sickness in man by atoxyl, and this has since been done by many observers (Kopke, Thiroux and d'Anfreville, Martin, van Campenhout, Koch and others) (5).

The results in delaying the rate of advance of the disease in the treated cases has been most gratifying, and treatment by atoxyl has now become a standard with all workers upon the subject, either alone or in conjunction with other remedies.

In one most important respect, however, progress has hitherto been lacking, and that lies in preventing a recurrence of the disease either on discontinuing the atoxyl treatment or, indeed, in preventing in a good many cases recurrence of the trypanosomes, even when dosing with atoxyl has been continued throughout the progress of the case.

Although there is some contradiction in the results obtained by different observers it may be said that the results on the whole obtained by treating sleeping sickness in man with atoxyl are very similar to those obtained by Thomas and Breinl in the treatment of animals experimentally infected with *T. gambiense.*

Just as in the case of animals, there appears to be a small percentage of cases in which after efficient treatment with atoxyl lapse does not take place, but in the great majority of cases which have been under observation for a sufficiently long period to form a judgment, recurrence takes place. After each recurrence the parasites can be driven out for a time with atoxyl but with increasing difficulty, and finally death occurs directly from trypanosomiasis.

In considering the permanent cure of the trypanosomiasis of *T. gambiense* in man, the tardy action of this parasite above alluded to must be borne in mind, and many of the cases recently reported as cured by atoxyl alone require longer watching before any definite conclusion can be drawn from them.

There are on the one hand undoubted cases where, in spite of continued treatment, relapses have kept on recurring, ending in the death of the patient, and on the other exceptional cases where after

much shorter treatment the trypanosomes have not reappeared and the cases as far as can be judged must be regarded as cured.

Similar exceptional cases due to some idiosyncrasies either in the animal or in the infection occur in every laboratory research, even on more rapidly fatal forms of trypanosomiasis such as nagana. Also in such cases even after months of waiting recurrence may sometimes take place. So that caution is necessary in all cases and safe conclusions can only be drawn by observing two conditions, viz., first, sufficiently long periods of treatment and watching; secondly, by the employment of the statistical method and avoiding judging from too few cases, or the behaviour of the exceptional cases.

To sum up the present position of our knowledge of the treatment of trypanosomiasis by atoxyl or the other remedies mentioned above. These substances are capable of temporarily causing the trypanosomes to disappear from the blood, and in a small percentage of cases reappearance is long delayed or may never occur; but in the vast majority of cases even when the parasites have been so caused to disappear for some time, reappearance takes place when treatment is stopped, or eventually, in many cases, even when treatment is continued throughout. The explanation of the apparent cures in sleeping sickness may be due chiefly to the more tardy appearance of the parasite characteristic of that affection, but even allowing all these cases there are recorded cases of persistent treatment with atoxyl for months in which the parasites continued in the blood and eventually produced death.

Several observers have used the different other trypanosome remedies in conjunction with the use of atoxyl with the object of avoiding the undesirable effects of prolonged use of arsenic in considerable quantities, and also to prevent the parasites becoming inured to the arsenic by holding them in check alternately with other non-arsenical drugs, such as the aniline compounds above mentioned.

Also, van Campenhout has used strychnine in conjunction with atoxyl, and a system of cold baths, in order to stimulate the depressed central nervous system. From this combination van Campenhout records very satisfactory results, although he does not claim that the

strychnine has any direct effect upon the parasites but that it supports and stimulates the central nervous system.

The combination or alternation of the aniline colours with atoxyl mentioned above does not appear to give much better results than atoxyl alone, as far as can be judged from the records. In all such cases two drugs have been used in combination or alternation which both act upon the ordinary active stage of the trypanosome. It does not appear to have occurred to previous observers to search for a drug which might be entirely inert upon this stage of the parasite, but might act upon the latent form of the trypanosome which must in all probability exist in blood or tissues while the active form is being held in abeyance by atoxyl or other drug (7).

This formed the starting point of our research, and we began our work by testing the effect of salts of other heavy metals than arsenic after the trypanosomes had first been driven out as completely as possible by one, or more, subcutaneous injections of sufficient doses of atoxyl.

A series of white rats were taken and inoculated as equally as possible with nagana, they were then observed daily until trypanosomes had appeared in large numbers in the blood of all members of the series; all members were then treated with atoxyl and the observations of the blood continued until all no longer showed trypanosomes in the blood. This stage was attained usually as the result of one injection of atoxyl but in most cases a second injection of atoxyl was given as a routine procedure in order to ensure complete disappearance of parasites in the usual or active form. When this position had been reached, the rats being used in the experiment were divided into two lots one of which received no further treatment and served as a control, while the other lot received injections three or four times of a solution of a salt of the particular heavy metal being tested after which treatment of this lot of animals also ceased. Observation was then kept up in all for reappearance of parasites in the blood, and the time of death of each animal was noted in cases where death occurred.

Working in this manner, salts of copper, lead and silver were tested without any positive results being obtained, but on testing

mercury in the form of mercuric chloride, a distinct and important positive result was fortunately obtained, the trypanosomes not reappearing in the majority of cases. A large percentage of the treated animals survived and are at present alive, several months now, in some cases, after full infection with a strong strain of nagana, while all the controls to all the experiments are now dead. In the case of two animals only from all our series was a recurrence of trypanosomes observed in spite of treatment with atoxyl followed by mercuric chloride.

In addition to the experiments on the action of mercury after atoxyl, we have carried out a series of experiments on the nature and chemical properties of atoxyl, with a view to a more rational knowledge of its mode of action and decomposition in the body, and the reason for its different action from certain other organic arsenic compounds such as the cacodyl compounds on the one hand and from the inorganic arsenic compounds on the other.

The experiments on treatment are given in Section A, those on the chemistry of atoxyl in Section B.

Section A

The Treatment of Experimental Nagana in White Rats by Atoxyl, followed by a Mercuric Salt

Both drugs were administered subcutaneously, in sterile aqueous solution, the atoxyl in 5 per cent. solution, the mercury salt as the liquor hydrargyri perchloridi B.P. containing 0·1 per cent. of mercuric chloride, or in one experiment as Donovan's solution (1 per cent. iodide of mercury and 1 per cent. of iodide of arsenic in water).

All the rats were inoculated subcutaneously with approximately 0·4 c.c. of blood taken from an infected rat on the second day of infection and mixed with sodium citrate solution.

The strains of *T. brucei* employed killed untreated rats in from five to seven days after inoculation, but in some of the later experiments a particularly virulent strain was used which killed rats in from two to three days.

This virulent strain was obtained from a rat infected by the ordinary strain used, in which trypanosomes had reappeared after treatment by atoxyl alone. This increase in virulence after treatment by atoxyl was more than once observed during our experiments, and it appears to us that this may assume a certain importance in regard to the treatment of trypanosomiasis by atoxyl alone, especially in the treatment of sleeping sickness.

The treatment by atoxyl was only commenced after the disease was well advanced, as shown by the presence of abundance of trypanosomes in the animal's blood.

It is of high importance that the solution of atoxyl should be prepared fresh immediately before use, as the substance undergoes rather rapid alteration, probably a hydrolytic change, on keeping in aqueous solution especially in the light. As a result one obtains with a kept solution more of the toxic action of inorganic arsenical preparations and less of the specific effects of the atoxyl. Both these effects are inimical to the obtaining of good results, the toxic action of the arsenic on the rat being increased and the destructive action on the trypanosomes being decreased. This change in an exaggerated degree is seen on injecting into rats very old atoxyl solutions, thus a solution which had been kept for seven months in the light caused, in amount which is safe with the fresh solution (0·5 c.c.), *death within four hours*, while a solution which had undergone somewhat less decomposition, having been kept for the same period in an amber-coloured bottle in a cupboard, caused death in 25 to 37 hours, when also given in 0·5 c.c. dose. Unfortunately this change in the atoxyl on keeping in aqueous solution was not known to us in the earlier part of our work, and we attribute some of the deaths occurring soon after treatment was begun to the toxic effect of inorganic arsenic from partially changed atoxyl.

Accordingly the precaution of only using freshly-made solutions may be recommended to those working with atoxyl in trypanosomiasis (6).

In regard to the mercury treatment, this must only be commenced after the trypanosomes in their usual well-known active form have been completely driven out of the blood.

It has been conclusively shown by the work of previous observers, and we have also taken the precaution to demonstrate by preliminary experiments of our own that mercury treatment *alone* will not affect the usual form of trypanosome in the blood.

It is after the trypanosomes have been driven out of the blood, or changed into some inert passive form that the mercury salts become effective. Hence atoxyl, or probably some other drug capable of causing the primary change must be used first, and *after* that the mercury salt.

It is also essential in order that good results may be obtained that the largest possible therapeutic dose of both the atoxyl and mercury salt should be employed.

The routine examination of the blood was made in fresh $\frac{3}{4}$-inch square coverslip preparations. Occasionally the blood of important animals was centrifugalised.

For purposes of comparison of the control rats, treated by atoxyl alone, with those treated by atoxyl followed by a mercuric salt, it may be stated here that, as a general rule, the *T. brucei* reappeared in the circulation of the rats treated by atoxyl alone in from sixteen to twenty-five days after the atoxyl was stopped.

Administration of atoxyl was commenced on the third to fifth day after inoculation, dependent on the time at which the parasites appeared in abundance in the blood. Subsequent treatment with the mercuric salt commenced on the eleventh day, dependent as above stated on the complete disappearance of the parasites from the blood.

For the first ten days or a fortnight after the completion of treatment the animal's blood was examined daily. As the animals lived longer the examinations became less and less frequent until they were done approximately weekly. The blood of any animal evidently ill was immediately examined and, if necessary, sub-inoculations were made.

Records of Experiments

Experiment I.—Four rats received 0·5 c.c. of a 5 per cent. solution of atoxyl on the eighth day after inoculation, the third day after trypanosomes had been observed in the blood. On the four following days 2 c.c. of the mercuric perchloride was given. *Trypanosomes were never afterwards seen in any of these four rats.* One died forty-two days after inoculation, cause of death not discoverable, but not accompanied by trypanosomes or showing symptoms of trypanosomiasis. A second, being moribund, was killed ninety-five days after inoculation. Trypanosomes were not seen at the autopsy on either animal, and a rat sub-infected from the rat killed has never shown trypanosomes. The remaining two rats of this experiment are still alive 181 days after inoculation. Rats and mice sub-inoculated from them on the 92nd and 156th days have never shown trypanosomes and are still alive.

Experiment II.—Six rats received 0·8 c.c. of the atoxyl solution in two doses on the second and third days after inoculation. The infection was severe; one rat died before, two others just after, the first dose of atoxyl. Two of the remaining three animals received no further treatment. One of these two died of trypanosomiasis in twenty-two days, the other in twenty-seven days after the cessation of treatment. In the sixth rat, the course of atoxyl was followed by 1·5 c.c. of mercury perchloride solution given in two doses on the fifth and eleventh days after inoculation. Trypanosomes never reappeared in this animal. Thirty-one days after inoculation the animal seemed ill; it was therefore killed and another rat was sub-inoculated. Trypanosomes never appeared in the sub-inoculated animal, which died of skin disease three months later.

Experiment III.—Five rats each received 0·5 c.c. of atoxyl solution on the third day after inoculation. Two of these rats received no further treatment; one died, cause of death not discoverable, sixteen days after inoculation, the other of trypanosomiasis in twenty-eight days, the parasites reappearing three days before death. In the remaining three rats the atoxyl was followed by 2·7 c.c. of mercury perchloride given in four doses on the fifth, seventh, tenth and eleventh days after inoculation. One died, no trypanosomes, in forty days, the remaining two are still alive 155 days after inoculation. Mice sub-inoculated from them 130 days after inoculation have not become infected.

Experiment IV.—Two rats each received 0·5 c.c. of atoxyl solution on the fifth day after inoculation. One had no further treatment and died three days later. The other received 1·9 c.c. of the mercury perchloride solution in three doses on the eighth, tenth and twelfth days after inoculation. *Trypanosomes reappeared on the fifteenth day.* Another 0·5 c.c. of the atoxyl solution was given at once but the animal died next day.

Treatment was probably commenced too late here; both animals were almost moribund when the atoxyl was given. It is possible that the result might have been better in the mercury-treated rat had a larger dose of atoxyl been given. One dose is probably insufficient for so heavily infected an animal. It is questionable whether the parasites were ever entirely absent from the circulation.

Experiment V.—Six rats were inoculated ; one died on the third day. On the fourth day the remaining five rats received 1 c.c. of the atoxyl solution in two doses. Two had no further treatment. Trypanosomes reappeared in these two rats in twenty and twenty-three days respectively after the cessation of atoxyl, and they died two days later of trypanosomiasis (thirty days after inoculation). The remaining three rats, after the atoxyl treatment, each received 2·7 c.c. of the mercury perchloride solution in four doses on the fifth to eighth days after inoculation. Trypanosomes were not again seen in any of the three. One died (spleen enlarged but direct cause of death unknown) twenty days after inoculation ; the other two are still alive 123 days after inoculation. Mice inoculated from them ninety-eight days after inoculation were not infected and are still alive.

Experiment VI.—Twelve rats were taken for experiment, four died during the night of the second day after inoculation. On the third and fourth days the remaining eight received 1 c.c. of the atoxyl solution in two doses. Four of these eight rats received no further treatment ; one died almost immediately ; parasites reappeared in the other three in from ten to forty-one days after the cessation of treatment, and all died in from sixteen to forty-nine days after inoculation.

On the fourth, eighth, tenth and eleventh days after inoculation 2·7 c.c. of mercury perchloride was given in four doses to each of the other four rats. One died after the first dose, all of the three others were negative until thirty-six days after inoculation *when trypanosomes reappeared in* ONE *of them.* The other two are still negative at eighty-four days after inoculation ; mice sub-inoculated from them at fifty-nine days after inoculation have not shown trypanosomes.

The rat in which trypanosomes reappeared received 1 c.c. of the atoxyl solution in two doses on the day its relapse was detected, and the second day after. It then received 1·7 c.c. of mercury perchloride in three doses on the fifth, seventh, and ninth days after the relapse. Parasites again reappeared in fourteen days after the relapse. Atoxyl was again immediately given, 1 c.c. in two doses on successive days ; the trypanosomes disappeared as usual from the peripheral circulation. The animal died unexpectedly seven days later. Trypanosomes had not reappeared in its peripheral blood ; spleen and lymphatic glands were enlarged.

This is the only instance of a recurrence of trypanosomes after a satisfactory combined treatment by atoxyl and mercury. In this experiment treatment was certainly commenced very late. There was a smaller interval between the administration of the two drugs than is usual ; but there were probably no infective trypanosomes left in the peripheral circulation after the atoxyl treatment since a rat sub-inoculated at that time has not since shown trypanosomes and still lives.

Experiment VII.—On the third and fourth days after inoculation ten rats received 1 c.c. of the atoxyl solution in two doses ; two rats died during the night of the third day. Four of the remaining eight received no further treatment. Trypanosomes reappeared

in them in from thirteen to twenty-four days after the cessation of treatment, and they died two to three days later.

The other four rats received 2·7 c.c. of mercury perchloride solution in four doses on the sixth to tenth days after inoculation; trypanosomes were not again seen in any of them. One died, cause unknown, twenty-five days after inoculation. The remaining three are still alive fifty-six days after inoculation, and mice sub-inoculated from them on the thirty-first day are alive and have not shown trypanosomes.

Experiment VIII.—Atoxyl followed by Donovan's solution. Eight rats received 1 c.c. of a 3 per cent. solution of atoxyl in two doses on the fourth and fifth days after inoculation. Two died during the fourth night. On the sixth, eighth, and tenth days 1·5 c.c. of Donovan's solution was given in three doses to the remaining six rats. All of them are still alive forty-four days after inoculation; mice sub-inoculated on the nineteenth day are alive and have not shown trypanosomes.

Summary of Experiments

The following tabular statement presents in a summarised form the main results of the experiments :—

Total number of rats used for experiments	53
Number of deaths before any treatment, or at commencement of atoxyl treatment	14
I. *Effect of treatment by atoxyl alone, the treatment being stopped when blood was free from trypanosomes, i.e., after one or two doses :—*	
(*a*) Number of rats treated	14
(*b*) Deaths from trypanosomiasis	12
(*c*) Deaths from unobserved or unknown causes ...	2
(*d*) Percentage of survivals in rats treated by atoxyl alone[1]	0
II. *Effect of treatment by mercury salt, after exactly the same atoxyl treatment as in rats in* I :—	
(*a*) Number of rats treated	25
(*b*) Deaths from trypanosomiasis	2
(*c*) Deaths from unobserved or unknown causes ...	4

1. Including other work not described in the text, we have treated in all 113 rats experimentally infected with *T. brucei* with a single dose of atoxyl alone; of these only three have survived.

(*d*) Rats killed when unhealthy or moribund but showing no trypanosomes, and giving no trypanosomiasis on sub-inoculation	2
(*e*) Number of survivals	17
(*f*) Percentage of survivals	68

Particular attention may be drawn to the contrast between survivals after atoxyl alone, and atoxyl followed by mercury. The somewhat high percentage of deaths before treatment was begun or just at the commencement of atoxyl administration may be ascribed to two causes, the first and most important of these being that we purposely only worked with animals showing high infection, and therefore began treatment at a very late stage, and secondly, in earlier experiments the point as to freshness of atoxyl solution was not sufficiently appreciated, and in some instances there was evidence that these deaths at an early stage were due to intoxication by the deteriorated solution in animals already enfeebled by the trypanosome infection.

In concluding this section we may make some suggestions as to the mode in which the mercury prevents recurrence of the trypanosomes. One possible view would be that atoxyl usually kills all the trypanosomes save some more resistant forms.[1] The parasites are left in a 'weakened state' in which they are destroyed by the ordinarily inert mercury. Such an hypothesis indicates the successive combined employment of various trypanocidal substances in the treatment of trypanosome infections.

It may be that the parasite weakened but becoming immune, accustomed to the first substance administered may be killed by the unaccustomed second or third substance given immediately afterwards. Thus atoxyl might on this view be alternated with trypanroth or the aniline colours Ph and Cl, as suggested by Mesnil and Nicolle.

The objection to this view, however, is that as already stated above mercury salts alone without primary driving out by atoxyl (or

1. This suggestion was originally made to us by Dr. Thomas.

possibly by other primary trypanocides) have no apparent effect, and some amount of amelioration would certainly be expected did the mercury ions attack trypanosomes of ordinary form enfeebled by atoxyl.

Further, there is no evidence that weakening by atoxyl would lead to attack by mercury. Again we have evidence from the results of infection from rats in which trypanosomes have recurred after atoxyl treatment that the strain is now considerably more virulent than at first, and hence the trypanosomes appear stronger instead of weaker.

Finally, the combined and alternate using of atoxyl and other primary trypanocides such as the aniline dyes (Ph and Cl), in the hands of Mesnil and Nicolle, have not given appreciably better results as to cure or prevention of recurrence than the use of atoxyl alone. We are accordingly disposed at the present stage in our work, not to regard the mercury salts as being a parasiticide for the ordinary usual forms of trypanosome found active in the blood; but instead that another distinct stage or stages exists for which atoxyl does not act as a parasiticide or at least not as an effectual one, and that the mercury salt destroys this stage, and by so doing effects (after all the well-known stage has been first destroyed by atoxyl) the complete removal of the parasite from the organism and so prevents recurrence.

For clearness, let us call the usual well-known phase of the trypanosome A, and the other hypothetical phase, for the probable existence of which a certain amount of histological evidence already exists, B (7). Let us suppose that atoxyl is poisonous to A, but does not touch B; mercury salts, as we know from mercury treatment alone, do not touch A; let us suppose that they are, however, poisonous to B. Now if A and B are two phases in the life history of the parasite,[1] they may exist side by side in the blood and tissues, or one in the blood and the other in the tissues of an infected individual.

1. This does not of course exclude the existence of other phases in the tsetse-fly or other carrier of the parasite.

If now effectual treatment by atoxyl be given A is completely destroyed, but B is not affected.

Indeed, as is often found to happen when the environment is made inimical to the existence of any given phase of an organism, the act of destroying A may cause many of the parasites of that phase to give rise to individuals of phase B (7).

If now, all of A having been driven out, a parasiticide for B, such as the mercury salt of our experiments, be given, then both forms are destroyed, the individual is cleared of infection, and if both clearances be complete recurrence without re-infection becomes impossible.

If, on the other hand, the second parasiticide be not given, as long as the presence of the first parasiticide (atoxyl in this case) in the blood and body fluids of the infected individual keeps up detrimental conditions for A, passage from B phase back into A phase will be inhibited, and any few individuals of B phase passing back into A will be destroyed. When, however, from interruption of treatment, the pressure or concentration of the parasiticide (atoxyl) in the blood and lymph falls below a certain level then the parasites resting in phase B become once more free to pass back into phase A, and this takes place with recurrence of active infection. Further, even if the pressure of the first drug or parasiticide (atoxyl) be kept up continuously, the parasite in the resting phase B is always present unattacked by this drug, and all the time there is a tendency to escape, and there probably is a slow escape back into phase A, the escaping individuals being at first destroyed. We have here, however, all the conditions for gradual inurement to an inimical environment, and for the production of a strain of the organism immune to the parasiticide.

The result will be to produce an ' atoxyl-fast ' organism which then resists an atoxyl treatment and persists in spite of continued manifestation of the drug.[1]

We do not desire to put this forward at the present time as more than a working hypothesis, but it at any rate possesses an interest as having led us to seek for a second remedy for the prevention of

1. Ehrlich has obtained such an ' atoxyl-fest ' form of trypanosome (see note, p. 324).

recurrence of the parasite along new lines, that is to say, among substances which we, and others, had shown to possess no direct effect upon the usual form (A) of the parasite.

The previous attempts had been to alternate two substances both poisons for phase A; our scheme was to alternate a poison for phase A with some drug which should attack whatever A possibly passed into, or which was associated with A, viz., phase B.

We would venture to suggest similar experimental therapeutics in the case of other diseases caused by protozoa—the attempt in the case of chronic malaria, for example, to find some drug capable of preventing recurrences, which might possess not the slightest effect similar to quinine as a first drug.

Neither atoxyl nor mercury may prove of avail with other protozoa, but the fact that most protozoa possess widely different phases in their life cycle should be borne in mind and that a drug which is deadly in one phase may be quite inert and harmless in another, and accordingly the search is indicated for a suitable drug for each phase.

Section B

Notes on the Chemical Composition and Chemical and Physical Properties of Atoxyl and on the Probable Mode of its Action

The substance sold under this protected trade name by the firm Lanolinfabrik Martinikenfelde, Berlin, is a white crystalline powder readily and completely soluble in water, much less soluble but completely soluble in absolute alcohol.

The process for its preparation has not been published, but it is stated to be an anilide of metarsenious acid and its formula given as $C_6H_5NH \cdot AsO_2$.

We have been unable to obtain from the substance, however, any of the reactions which might be expected by analogy to occur with a body of such a constitution.

For example, it is not decomposed on warming with caustic alkalies as are most known anilides. It is not an ordinary salt of

aniline, as no aniline is set free on the addition of cold caustic alkali to its solution. Further, even on boiling with 40 per cent. caustic soda it does not distil off aniline readily as the well-known anilides all do. Long boiling with the strong alkali gives *traces* of aniline in the distillate as shown by the usual colour tests.

On dry distillation it gives a small yield of aniline.

It hydrolyses *slightly* more readily on long boiling in strongly acid solution. When boiled with fairly strong hydrochloric acid (about 2N was used) it gives a *slight* precipitate of As_2S_3 on passing sulphurretted hydrogen through the boiling solution. But with more dilute acid, even on boiling, only a precipitate of sulphur is obtained.

This marked stability to alkalies and acids stands in marked contrast to its fairly rapid hydrolysis on standing at ordinary temperatures in aqueous solution, already alluded to in Section A.

Laveran (6) has shown that this hydrolysis proceeds much more rapidly on superheating in aqueous solution in an autoclave to 120° C.

These reactions indicate that the arsenic is much more stably held than if it were attached in a side chain as shown in the formula quoted above. The only known organic arsenical compounds showing a similar stability to the action of alkalies are those *containing the arsenic directly united to the benzene ring*, such as phenyl-arsenic acid and its salts [C_6H_5 . As $(OH)_2$, and C_6H_5 As (OH) (O Na)].

Both the physical properties of the aqueous solution and the analyses for arsenic and nitrogen which we have been able to make also throw doubts upon the formula assigned to it. Also, on incineration and ignition, a considerable amount of sodium carbonate is left behind, too large to be present as an impurity, and strongly indicating that the compound is a sodium salt of an organic arsenical compound.

Physical Properties of the Aqueous Solution

Two determinations of the freezing point in aqueous solutions of 5 per cent. and 3 per cent. gave values for Δ of 0·606 and 0·367 respectively, leading to molecular weights of 153 and 151 respectively.

This low value for the molecular weight in aqueous solution points at once to electrolytic dissociation.[1]

On making determinations of the electrical conductivity we found this confirmed, the solution is a very good conductor, the 5 per cent. solution giving a resistance of 76 ohms, in a vessel in which $\frac{N}{10}$ KCl gave a resistance of 53·5 ohms.

This proves the important fact in the consideration of how the atoxyl produces its therapeutic effects, that the substance is highly electrolytically dissociated, and that the activity is in all probability due to an ion which contains arsenic in organic combination, and not as might have been supposed to inorganic arsenic slowly set free from a very feebly dissociated compound, as the result of slow hydrolysis in the body.

The electrical conductivity is also opposed to the view of an anilide composition for the compound.

Boiling Point of Alcoholic Solutions

An attempt was made to obtain the molecular weight free from the disturbing influence of electrolytic dissociation by making determinations of the rise of boiling point of solutions in absolute alcohol. It was found, however, that in alcohol the substance is in colloidal solution, no measurable rise in boiling point being found even in saturated solution.

Effects of Incineration

One gramme of the atoxyl was taken and heated on platinum, at first over a Bunsen, and then to bright redness with the blowpipe flame. Afterwards it was twice moistened with strong hydrochloric acid, the residue extracted with distilled water and the amount of chloride determined.

On first heating with the Bunsen it commences to char without previously melting or volatilizing. As it chars it gives off arsenical fumes, and a cacodyl-like odour is obtained; after this first incineration

1. Taking the molecular weight as 239 (*vide infra*), the Δ obtained above leads to an ionization of 58·2 per cent., assuming that ionization into two ions occurs.

the weight of residue was 0·37 gramme (weight of atoxyl taken, 1 gramme); on moistening with distilled water, and then heating to bright redness for some minutes over the Bunsen, the weight of residue was 0·274 gramme. At this stage a very brilliant sodium flame was obtained.

The residue was moistened with hydrochloric acid and a very brisk effervescence was obtained showing the presence of a carbonate of a fixed alkali. On reheating a splendid sodium flame was obtained, and more As_2O_3 was given off. The heating after re-moistening with hydrochloric acid was repeated, and the residue was finally heated to bright redness in the blowpipe flame when it fused. The final weight of the residue was 0·224 gramme = 22·4 per cent. of the atoxyl taken originally.

These results appear to us to show unmistakably that atoxyl is a sodium salt. If the 22·4 per cent. of residue obtained above be taken as being sodium chloride this leads to 8·8 per cent. of sodium in the original atoxyl.[1]

Determinations of Nitrogen

The percentage of nitrogen was estimated in two Kjeldahl determinations and one by Dumas' method.

The two Kjeldahl determinations gave 4·7 and 4·9 per cent. of nitrogen respectively, and the Dumas gave 4·83 per cent. Taking 4·8 per cent. as an average, this gives a molecular weight of 292 for one nitrogen atom.

It may be pointed out that these nitrogen determinations which are concordant among themselves are quite different from the percentage required by the reputed formula representing atoxyl as an anilide.

1. In a subsequent experiment made expressly to determine exactly the percentage of sodium by incinerating in presence of excess of sulphuric acid to drive off all the arsenic, from 1 gram of atoxyl a weight of 0·2184 of dry Na_2SO_4 was obtained giving 7·1 per cent. of sodium. The sulphate was then precipitated as $BaSO_4$ and the percentage of sodium calculated from the weight of this precipitate gave 7·5 per cent. Taking 7·5 as the percentage of sodium, the molecular weight for one sodium molecule works out to 307.

Determinations of Arsenic

These were first attempted by heating with strong nitric acid in sealed tubes by Carius's method, but erroneous results were obtained probably due to the stability of the compound causing incomplete breaking up by the fuming nitric acid. Later the method of fusing with caustic alkali was employed, followed by determination of the arsenic as $Mg_2As_2O_7$. This method was suggested to us by the fact that the analogous phosphorus compound to what we suspected atoxyl to be, viz., amido-phosphenylic acid, breaks up readily on fusing with alkalies into aniline and phosphoric acid.[1]

In the analysis the weight of atoyxl taken was 0·6476 gramme, the weight of the $Mg_2As_2O_7$ precipitate was 0·3987, yielding 29·65 per cent. of arsenic. This gives a molecular weight of 253 for one atom of arsenic.

Analysis of the Silver Salt

The percentage of silver in the silver salt produced by precipitation of the aqueous solution by silver nitrate solution, was also estimated by the usual method for organic silver salts, and gave as the mean of two determinations 35·83 per cent. leading to a molecular weight for one atom of 302 for the dry silver compound and of 217 for the dry sodium salt (atoxyl) or adding for the percentage of water (*vide infra*) = 271.

Behaviour with Salts of Heavy Metals

Atoxyl forms insoluble salts with the heavy metals such as silver and copper. On addition of a solution of silver nitrate to a solution of atoxyl in distilled water a heavy precipitate, pure white in colour, is obtained not soluble in excess of the silver nitrate but readily soluble in either ammonia or nitric acid. A solution of copper sulphate gives a canary-yellow precipitate, which is also soluble in either ammonia or nitric acid. The colour of the precipitate with silver nitrate shows the absence of either arsenious or arsenic acids. Phenyl-arsenic acid gives similar insoluble salts with silver and copper.

Since atoxyl gives aniline as a decomposition product on hydrolysis in aqueous solution, we are inclined to regard it, from this and the

1. It was found later that a rapid and accurate method of determining the arsenic consists in destroying by a mixture of sulphuric and nitric acid (10 c.c. of sulphuric and 5 c.c. of nitric to 1 gramme of substance) in a Kjeldahl flask, neutralizing with ammonia, precipitating with magnesia mixture, collecting on a Gooch filter, and weighing as $Mg_2As_2O_7$. Analysis by this method gave 26·2 per cent. = mol. wt. of 286.

above evidence, as in all probability a sodium salt of amido-phenyl-arsenic acid or some derivative of such an acid, containing the arsenic directly united to the benzene ring.

We are at present engaged in attempts to synthesize this and similar arsenical compounds, with a view to testing their efficiency as parasiticides for trypanosomes and other parasitic protozoa.

Mode of Therapeutic Action of Atoxyl

It is of high scientific interest to consider how atoxyl produces its therapeutic action.

At first from its published formula as an anilide, we were inclined to think that this was very feebly ionized in solution so as to yield a low pressure or concentration of the arsenical ion, and that this pressure, without becoming at any time so high as to produce poisonous results, was maintained for a long time after the atoxyl had been administered by the large non-ionized portion of the anilide becoming slowly ionized as the arsenical ion of the ionized portion became used up by trypanosomes and tissues.

On this view, the difference in therapeutic action between inorganic arsenical compounds and an anilide such as atoxyl is described to be would have lain in the fact, that the concentration attainable with the inorganic compound being sharply limited by its toxic action, much inorganic arsenic cannot be given and the initial concentration very rapidly falls off; on the contrary in the feebly ionised organic anilide there would be a bank to draw upon in the large non-ionized fraction, and with such a dose as would give the same initial concentration in arsenical ion, the drop afterwards would be infinitely slower so that a sustained effect would be obtained without poisoning the animal treated. Also, the difference between one organic compound and another as between the ineffectual cacodylates and the atoxyl might have found an explanation in difference in dissociation, the cacodylates being so feebly dissociated as not to be capable of acting on the trypanosomes.

We must admit, however, that our work on the chemistry of atoxyl and its physical properties has caused us reluctantly to abandon

this simple explanation, and come to the conclusion that the action is a specific one due to a peculiarly constituted organic arsenic-containing ion.

The chemical considerations have shown that the fresh solution contains its arsenic very strongly attached and that aniline is not readily detached, and this view is supported by the physiological action of the drug when pushed; as has been shown by other observers. The poisoning effects with a large dose are neither those of arsenical poisoning nor of aniline poisoning. Nor with long continued use are the chronic effects of arsenic on the nervous system obtained, nor the anaemia, nor effects on blood corpuscles of a haemolytic agent such as aniline. The toxicity of atoxyl is only about $\frac{1}{80}$th of that of arsenic, and as just stated even then the symptoms are not those of acute arsenical poisoning; nor is there the action of free aniline, a much less amount of aniline than that contained in the atoxyl causes acute poisoning.

But these results cannot be explained on the basis of feeble dissociation, for as shown above by the electrical conductivity, atoxyl is very highly dissociated.

Hence it appears to us that the action is not to be ascribed either to an inorganic arsenical ion, or aniline, slowly formed by decomposition of the atoxyl, *but to direct and specific action of a complex organic ion containing both the aniline and arsenical groups.*

This view is in our opinion supported by the rapidity with which the atoxyl acts, a single dose given to a highly infected animal may cause entire absence of parasites from the blood on the following morning.

This exceedingly rapid action is hardly compatible with a slow decomposition of the drug.

Conclusions

1. Parasitic protozoa which show different phases in their life history may be attackable at one phase by a drug which is entirely inert and therapeutically useless at another phase, and conversely a drug which is without action on the first phase may be specific in its action upon the second phase.

2. For this reason, in the study of experimental therapeutics applied to protozoan parasites, not merely one drug must be tried, or an alternation of two drugs which have a lethal effect upon the parasite at one and the same stage only, but rather a drug which kills at one stage having been discovered; for the prevention of recurrence a second drug should be sought out which will attack the parasite in the succeeding stage, and this drug may not be found amongst others which kill at the first stage, but may well be sought outside that circle.

3. A special study has been submitted of atoxyl treatment followed by a mercury salt in rats infected by nagana (*T. brucei*), and it appears that such combined treatment gives better results than uncontinued treatment by atoxyl alone, although mercury salts alone have no action on the trypanosome.

4. It is suggested that the combined treatment should be given a careful trial in natural trypanosome infections of man and animals.

5. The treatment must be commenced as soon in the infection as possible; full therapeutic doses of both drugs must be given; fresh solution of atoxyl must be used; and the mercury salt begins to take effect when the parasites have been driven from the circulation by the atoxyl.

6. The substance atoxyl is in all probability not an anilide, but a sodium salt of an organic acid containing an amidogen group, and an arsenic radical directly united to a benzene ring.

The aqueous solution is strongly electrolytically dissociated, giving in consequence an apparently low molecular weight by the freezing point method, and possessing a high electrical conductivity.

Except on standing in aqueous solution, it is a most stable compound, and neither aniline nor arsenic are easily detachable from its molecule by chemical means.

Its toxic properties are neither those of arsenic nor of aniline even when pushed to excess, and its therapeutic action is rapid, from this and its high conductivity showing high dissociation, the conclusion is drawn that its activity must be ascribed not to free inorganic arsenical ions or to free aniline, but to a complex organic ion containing both the arsenical and aniline radicals.

Atoxyl forms insoluble salts with silver and copper, which are insoluble in neutral solution but soluble in ammonia and in nitric acid.

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LITERATURE

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Note added May 6th, 1907.—After our paper was concluded we received a reprint from Professor Ehrlich (*Chemo.-therapeutisch Trypanosomen-studien, Berl. klin. Wochensch.*, 1907, Nos. 9-12) in which he, in collaboration with Bertheim, has established that atoxyl is the sodium salt of para-amido-phenyl-arsenic acid, with four molecules of water of crystallization. Our analyses strongly support such a view except that we have found a somewhat lower amount of water.

A determination of the loss of weight on heating to a constant weight at 145°C. for some hours gave a loss of weight of 0·1984 grammes in 1·001 grammes of atoxyl, giving a percentage of 19·81 of water. This comes to slightly over three molecules of water of crystallization instead of four to the molecule of sodium-amido-phenyl-arseniate. The amount of water in commercial samples of atoxyl probably varies somewhat. Our analytical figures as well as the reactions and physical properties of atoxyl, as above stated, agree so closely with those which a salt of amido-phenyl-arsenic acid would show that we regard them as confirmatory of the view of Ehrlich and Bertheim.

The formula $NH_2(C_6H_4)AsO \cdot ONa \cdot OH, 3H_2O$ requires H_2O = 18·43, As = 25·6, N = 4·77, Na = 7·89, we have found H_2O = 19·81, As = 26·2, N = 4·8, Na = 7·5 per cent.